

CLAIMS:

1. A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant Δ^9 -18:0-ACP desaturase having one or more amino acid substitutions selected from the group consisting of:

- a) Ala, Thr, Ser, or Ile at the residue homologous to Met 114 of SEQ ID NO: 1;
- b) Arg at the residue homologous to Thr 117 of SEQ ID NO: 1;
- c) Gly, Ala or Cys at the residue homologous to Leu 118 of SEQ ID NO: 1;
- d) Val or Leu at the residue homologous to Pro 179 of SEQ ID NO: 1;
- e) Val, Ser, Phe or Trp at the residue homologous to Thr 181 of SEQ ID NO: 1; and
- f) Leu or Thr at the residue homologous to Gly 188 of SEQ ID NO: 1.

2. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes each of the following amino acid substitutions:

a) Ala at the residue homologous to Met 114 of SEQ ID
NO: 1;

b) Arg at the residue homologous to Thr 117 of SEQ ID
NO: 1;

c) Gly at the residue homologous to Leu 118 of SEQ ID
NO: 1;

10 d) Val at the residue homologous to Pro 179 of SEQ ID
NO: 1;

e) Val at the residue homologous to Thr 181 of SEQ ID
NO: 1; and

f) Leu at the residue homologous to Gly 188 of SEQ ID
NO: 1.

3. The DNA expression construct of Claim 1 in which
the nucleic acid sequence encodes each of the following
amino acid substitutions:

a) Thr at the residue homologous to Met 114 of SEQ ID
NO: 1;

b) Arg at the residue homologous to Thr 117 of SEQ ID
NO: 1;

c) Ala at the residue homologous to Leu 118 of SEQ ID
NO: 1;

10 d) Leu at the residue homologous to Pro 179 of SEQ ID
NO: 1;

e) Ser at the residue homologous to Thr 181 of SEQ ID NO: 1; and

f) Leu at the residue homologous to Gly 188 of SEQ ID NO: 1.

4. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes each of the following amino acid substitutions:

a) Ser at the residue homologous to Met 114 of SEQ ID NO: 1;

b) Arg at the residue homologous to Thr 117 of SEQ ID NO: 1;

c) Cys at the residue homologous to Leu 118 of SEQ ID NO: 1;

d) Leu at the residue homologous to Pro 179 of SEQ ID NO: 1; and

e) Thr at the residue homologous to Gly 188 of SEQ ID NO: 1.

5. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes the amino acid substitutions Arg at the residue homologous to Thr 117 and Leu at the residue homologous to Gly 188 of SEQ ID NO: 1.

6. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes the amino acid

substitution Arg at the residue homologous to Thr 117 of SEQ ID NO: 1.

7. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes the amino acid substitution Phe at the residue homologous to Thr 181 of SEQ ID NO: 1.

8. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes the amino acid substitution Trp at the residue homologous to Thr 181 of SEQ ID NO: 1.

9. The DNA expression construct of Claim 1 in which the nucleic acid sequence encodes the amino acid substitutions Ile at the residue homologous to Met 114 and Leu at the residue homologous to Gly 188 of SEQ ID NO: 1.

10. The DNA expression construct of any one of Claims 1, 2, 3, 4, 5, 6, 7, 8, or 9 wherein the nucleic acid sequence is selected from the Δ^9 -18:0-ACP desaturase sequences from a member of the group consisting castor, brassica, sunflower, yellow lupine, cotton, coriander, maize, sesame, rice, flax, safflower, avocado and cucumber.

11. A cell transformed with the DNA expression construct of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

12. The cell of Claim 11 which is a prokaryotic cell.

13. The cell of Claim 11 which is an eukaryotic cell.

14. The cell of Claim 13 which is a plant cell.

15. A transgenic plant expressing the DNA construct of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

16. The transgenic plant of Claim 15 which is *Arabidopsis thaliana*.

17. The transgenic plant of Claim 15 which is selected from the group consisting of castor, brassica, sunflower, yellow lupine, cotton, coriander, maize, sesame, rice, flax, safflower, avocado and cucumber.

18. A method for specifically altering a function of a protein through directed mutagenesis, comprising:

- a) identifying candidate amino acids of the protein which when replaced by different amino acids are predicted to alter the function of the protein;
- b) generating a library of mutated DNA sequences encoding the protein, the mutated sequences being generated by simultaneously randomizing the codons for every candidate amino acid of step a); and
- 10 c) identifying proteins encoded by the library of step b) which exhibit the desired specific alteration of function.

19. The method of Claim 18 wherein the candidate amino acids are identified by a combination of methods.

20. The method of Claim 18 wherein the candidate amino acids comprise amino acids which directly participate in the function which is to be altered.

21. The method of Claim 20 wherein the candidate amino acids further comprise amino acids which indirectly participate in the function which is to be altered.

22. The method of Claim 18 wherein the candidate amino acids are identified by random mutagenesis.

23. The method of Claim 18 wherein the candidate amino acids are identified by structural analysis of the protein.

24. The method of Claim 18 wherein the candidate amino acids are identified by sequence analysis and comparison to related proteins.

25. The method of Claim 18 wherein the library of mutated DNA sequences is generated by overlap extension PCR.

26. The method of Claim 18 wherein proteins which exhibit the desired alteration of function are identified by a selective screening process.

27. The method of Claim 18 wherein the protein is an enzyme.

28. The method of Claim 27 wherein *in vivo* activity of the enzyme is altered.

29. The method of Claim 27 wherein *in vitro* activity of the enzyme is altered.

30. The method of Claim 27 wherein *in vivo* and *in vitro* activity of the enzyme is altered.

31. The method of Claim 27 wherein substrate specificity of the enzyme is altered.

32. The method of Claim 31 wherein the enzyme is an 18 carbon atom-specific fatty acid desaturase enzyme.

33. The method of Claim 32 wherein the fatty acid desaturase is a plant fatty acid desaturase.

34. The method of Claim 33 wherein said library of mutated DNA sequences is generated from an 18:0 desaturase DNA sequence obtained from one of the group consisting of castor, brassica, sunflower, yellow lupine, cotton, coriander, maize, sesame, rice, flax, safflower, avocado and cucumber.

35. The method of Claim 33 wherein the plant is selected from the group consisting of castor, brassica, sunflower, yellow lupine, cotton, coriander, maize, sesame, rice, flax, safflower, avocado and cucumber.

36. The method of Claim 33 wherein the candidate amino acids are homologous to amino acid residues 114, 117, 118, 179, 181 and 188 of castor Δ^9 -18:0-ACP desaturase.

37. The method of Claim 33 wherein the desaturase is castor Δ^9 -18:0-ACP desaturase.

38. The method of Claim 32 wherein the substrate specificity alteration is a substantial increase in activity toward fatty acid substrates with chains containing fewer than 18 carbon atoms.

39. The method of Claim 38 wherein desaturase enzymes having said specific alteration are identified by the additional steps of:

- d) transforming the library of sequences of step b) into appropriate unsaturated fatty acid auxotroph host cells;
- e) culturing the transformed cells under selective conditions which conditions are also appropriate for expression of said sequences; and
- 10 f) separating and culturing individual isolates of the transformed cells that grow under selective conditions of step e) thereby identifying desaturase enzymes having the specifically altered substrate specificity.

40. The method of Claim 39 wherein the fatty acid auxotroph host cell is characterized as having an unaltered fatty acid profile following introduction of an 18 carbon atom-specific desaturase into the cell and having an altered fatty acid profile following introduction of a 16:0

desaturase, a 14:0 desaturase, a 12:0 desaturase or a 10:0 desaturase into the cell.

41. The method of Claim 40 wherein the fatty acid auxotroph host cell is MH13 *E. coli*.

42. A library of mutated DNA sequences generated by the method of Claim 33.

43. A mutant fatty acid desaturase enzyme encoded by a member of the library of Claim 42.

44. A mutant fatty acid desaturase enzyme identified by the method of Claim 39.

45. The fatty acid desaturase of Claim 44 which is derived from a castor Δ^9 -18:0-Acyl-ACP desaturase.

46. The fatty acid desaturase of Claim 45 having one or more of the following amino acid substitutions:

- a) Ala, Ser, Ile or Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly, Cys or Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Val or Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Val, Phe, Trp or Ser for Thr at residue 181 of SEQ ID NO: 1; and
- f) Leu or Thr for Gly at residue 188 of SEQ ID NO: 1.

47. The fatty acid desaturase of Claim 46 selected from the group consisting of:

- i) a desaturase having each of the following substitutions: M114A, T117R, L118G, P179V, T181V and G188L;
 - ii) a desaturase having each of the following substitutions: M117T, T117R, L118A, P179L, T181S and G188L;
 - 10 iii) a desaturase having each of the following substitutions: M114S, T117R, L118C, P179L and G188T;
 - iv) a desaturase having the substitutions: T117R and G188L;
 - v) a desaturase having the substitution: T117R;
 - vi) a desaturase having the substitution: T181F;
 - vii) a desaturase having the substitution: T181W;
 - viii) a desaturase having the substitutions: T117R and P179L; and,
 - ix) a desaturase having the substitutions: M114I and G188L.
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48. The method of Claim 18 wherein the protein is a ligand binding protein.

49. The method of Claim 48 wherein the *in vivo* ligand binding specificity of the protein is altered.

50. The method of Claim 48 wherein the *in vitro* ligand binding specificity of the protein is altered.

51. The method of Claim 48 wherein the *in vivo* and *in vitro* ligand binding specificity of the protein is altered.

52. The method of Claim 18 wherein the protein is a structural protein.

53. A library of mutated DNA sequences generated by the process of Claim 27.